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A new *Drechslerella* species isolated from *Orbilia* cf. *orientalis*

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Abstract — A new species of the nematode-trapping anamorphic genus *Drechslerella*, *D. yunnanensis*, was isolated from an unidentified *Orbilia* species. *Drechslerella yunnanensis* is characterized by unbranched or occasionally branched conidiophores with conspicuous denticles at the tip and non- to one-septate ellipsoidal conidia. In the presence of nematodes it forms constricting rings.

Key words — nematophagous fungi

Introduction

Subramanian (1964) erected *Drechslerella* for *D. acrochaeta* (Drechsler) Subram. 1964 based on the filiform apical appendage on its conidia. Although Liu & Zhang (1994) synonymized *Drechslerella* with *Monacrosporium* Oudem., detailed molecular analyses (Hagedorn & Scholler 1999) convinced Scholler et al. (1999) to propose new generic concepts for orbiliaceous nematode-trapping fungi based on mode of trapping device. We accept these concepts and recognize *Drechslerella* as characterized by forming three-celled constricting ring traps.

Two *Drechslerella* species have been linked thus far to known teleomorphs, both in *Orbilia: D. polybrocha* (Drechsler) M. Scholler et al. 1999 connected to *Orbilia tenebricosa* (Svrček) Baral 2006 and *D. brochopaga* (Drechsler) M. Scholler et al. 1999 connected to *O. orientalis* (Raitv.) Baral 1999 (Pfister 1997, Yu et al. 2006). With our new *Drechslerella* species from China, we describe a third *Drechslerella-Orbilia* connection.

Materials and methods

Collection of teleomorph, isolation and characterization of the anamorph

Fresh fruitbodies of an unidentified *Orbilia* sp. were collected by the first author in August 2006 from decaying bark of a broad-leaved tree, Dalongkou Park of Yimen County, Yunnan Province, China (N24°34', E101°00', alt. 1580 m, coniferous-broadleaf

forest dominated by *Cyclobalanopsis glaucoides* Schottky. and *Pinus armandii* Franch.). A dried voucher specimen was deposited in the Laboratory for Conservation and Utilization of Bio-resource, Yunnan Province, China (YMFT 1.01863). Anamorph were isolated by fixing several apothecia to a Petri dish lid of with their hymenia upside down to shoot ascospores on the surface of CMA (20 g corn meal, 18 g agar, 40 mg streptomycin, 30 mg ampicillin, 1000 ml distilled water) according to Yu et al. (2006). Morphological characters of both teleomorph and anamorph were observed and measured with an Olympus B51 microscope with differential interference contrast and a Zeiss Standard 20 microscope. Trapping organs were induced by adding ~100 nematodes (*Panagrellus redivivus* Goodey) into a 1 cm^2 slot formed by removal of the agar at the colony margin.

DNA extraction, PCR, and sequencing

Total DNA was isolated from fresh mycelium as described by Turner et al. (1997). Primer pairs ITS4 and ITS5 (White et al. 1990) were used to amplify the complete ITS (including 5.8S). PCR amplification parameters followed Yu et al. (2007). The PCR products were purified with a commercial Kit (Bioteke Biotechnology Co., Ltd., China) and sequenced on both strands with the same primers that were used for amplification with the aid of a LI-COR 4000L automatic sequencing system, using cycle sequencing with the ThermoSequenase-kit as described by Kindermann et al. (1998).

Phylogenetic analysis

We performed a phylogenetic analysis using ITS sequences of *D. yunnanensis*, morphologically similar species and some species of *Arthrobotrys* Corda and *Dactylellina* M. Morelet. (See Plate 3 for Genbank accession numbers).

DNA sequences were aligned using Clustalx 1.83. Parsimony analysis was run in PAUP* 4.0b10 (Swofford 2002). Cladistic analyses using the neighbor-joining method were performed with MEGA version 2.1. The neighbor-joining tree was constructed with Kimura 2-parameter model, including transitions and transversions with pairwise gap deletion.

Taxonomy

Anamorph

Drechslerella yunnanensis Z.F. Yu & K.Q. Zhang, **sp. nov.** Plate 1

MycoBank MB 512613

Coloniae in agaro albidae, post 10 dies 25°C ad 25 mm diam. Mycelium sparsum, hyphis septatis, 3.5–4 µm latis. Conidiophorae hyalinae, septatae, erectae, 60–220µm altae, basi 3.8–4.2 µm crassae, sursum leviter fastigiatae, apice 1.5–2 µm crassae, ibi ex denticulis brevibus obtusis saepius 2–7 conidia in capitulum pulchrum radians aggregate ferentes vel subinde usque 10 conidia in parte superiore rarius digesta gerentes. Conidia recta, elongate-ellipsoidea, apice rotundata, basin versus paulo attenuata, 7.8–12.9 longa, 3.3–4 µm crassa.

Holotype: YMF 1.01863, permanent slide, Yimen County, DaLongkou Forest Park, Yunnan Province, PR China, Ze Fen Yu, Aug. 2006.

Teleomorph (origin of isolate)**:** YMFT 1.01863, *Orbilia* cf. *orientalis*, collected on decayed bark of broad-leaved branch.

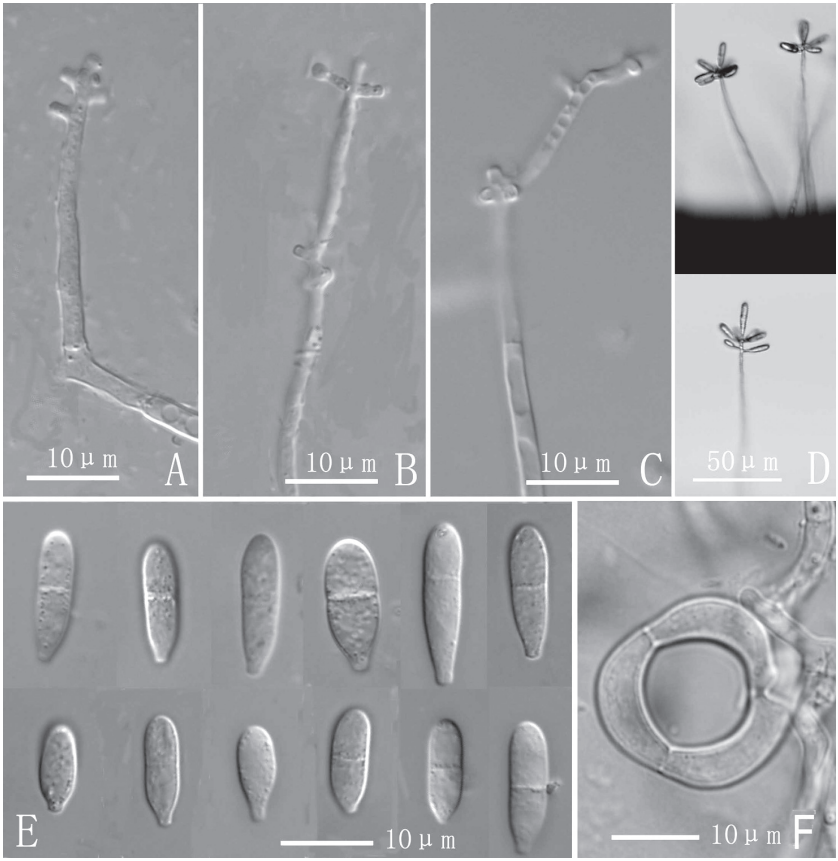

Plate 1. *Drechslerella yunnanensis* (YMF 1.01863) A–C. Conidiophores with short denticles. D. Conidiophores bearing conidia in clusters. E. Conidia. F. Constricting rings.

Etymology: The species epithet refers to the collection site of the teleomorph.

Colonies white, slow-growing on CMA medium, attaining less than 25 mm diam. in 10 days at 25°C. Vegetative hyphae hyaline, septate, 3.5–4 μm wide, aerial mycelium sparse, hyaline, septate, branched, 2.5–4 μm wide. Conidiophores hyaline, septate, erect, unbranched or occasionally branched below, 60–100(–220) μm, 3.8–4.2 μm wide at the base, tapering gradually upward to 1.5–2 μm near the tip, with 2–7(–10) denticles 2.3–4.2 μm long, each bearing one condium in a capitate or racemose arrangement. Conidia hyaline, straight, elongate ellipsoidal, rounded at the apex, with a small truncate protuberance at the base, 7.8–12.9(–17.8) × 3.3–4.2 μm, (0–)1 septate, proportion of aseptate conidia 17%. Nematodes are captured by means of stiped three-celled constricting

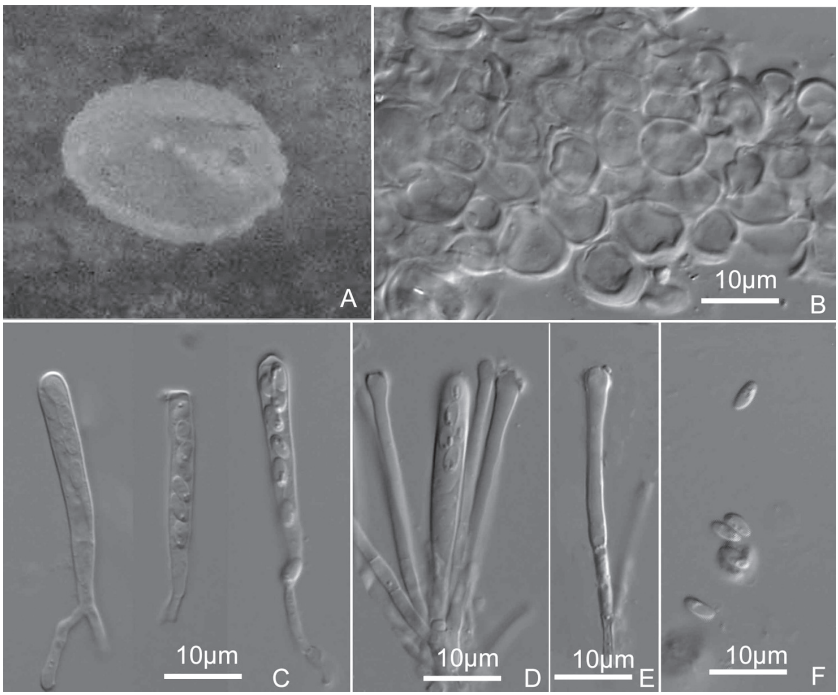

Plate 2. *Orbilia* cf. *orientalis* (YMFT 1.01863) A. Apothecium. B. Cells of excipulum. C. Asci. D. Cluster of asci and paraphyses. E. Paraphyses. F. Ascospores.

rings. In the non-constricted state, outer diameter measures 18–21.9 μm, the inner one 12–13.6 μm.

Teleomorph

Orbilia cf. ***orientalis*** Plate 2

Phylogenetic analysis

A neighbor-joining tree (plate 3) was constructed based on sequences of the ITS region of *D. yunnanensis* and other nematode-trapping fungi, with a non-trapping fungus, *Dactylella clavata* as outgroup. The phylogenetic tree shows the nematode-trapping fungi forming a monophyletic clade that comprises the three subclades corresponding to their trapping devices *Arthrobotrys* (adhesive networks), *Dactylellina* (adhesive knobs), and *Drechslerella* (constricting rings). In the *Drechslerella* clade, *D. yunnanensis* clusters as a species separate from *D. anchonia* (Drechsler) M. Scholler et al. 1999, *D. brochopaga,* and *D. dactyloides* (Drechsler) M. Scholler et al. 1999.

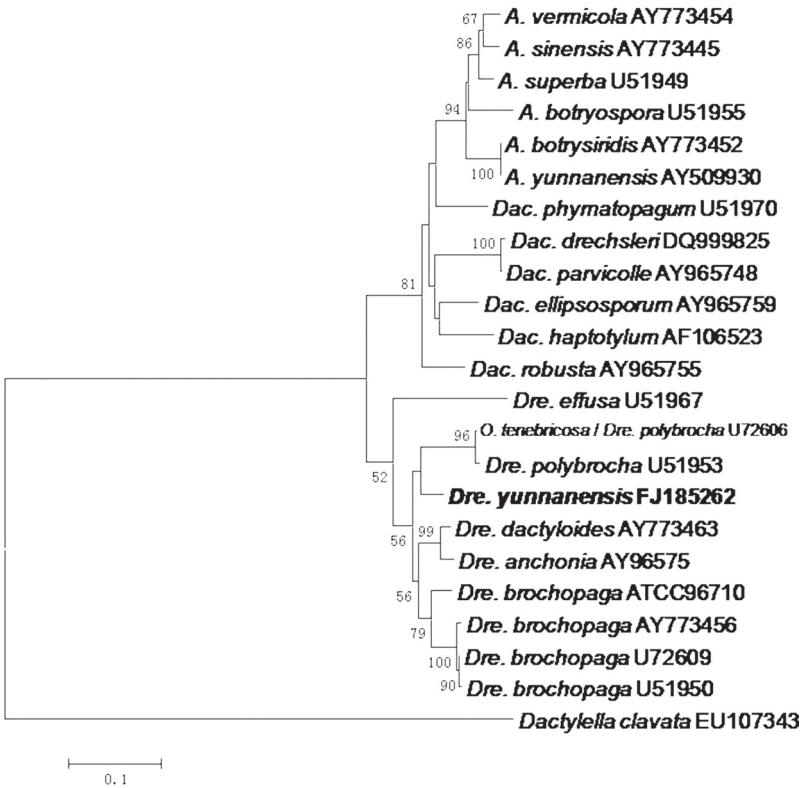

Plate 3. Phylogenetic tree of *Drechslerella yunnanensis* and related species using the neighbor-joining method based on ITS region sequence data. *Dactylella clavata* was used as outgroup. Bootstrap values less than 50% are not shown.

Discussion

Among the more than 80 nematode-trapping fungal species with trapping devices are 13 known *Drechslerella* species (Scholler et al. 1999), ten of which form only a single terminal conidium on each conidiophore. The most similar species to *D. yunnanensis* in morphology are *D. anchonia*, *D. brochopaga* and *D. dactyloides,* all of which have more than one conidium at the tip of the conidiophores in a capitate arrangement.

In addition, *D. brochopaga* has much larger conidia (26–46 × 5–9 μm) with more (mainly 3) septa (Drechsler 1937), and *D. anchonia* has obovoid, much larger conidia (29–43(–35) × 15–19(–16.8) μm) with a single septum in the lower third (Drechsler 1954). Conidia of *D. dactyloides* are narrowly ellipsoidal

or somewhat digitiform, often very slightly curved, with more conidia in the apex of conidiophores, and much larger (32–48 × 7–9.5 µm; Drechsler 1937).

The teleomorph of *D. yunnanensis* is very similar to *O. orientalis* in having cylindric-ellipsoid ascospores with a refractive, rod-shaped, laterally arranged spore body (SB) at the upper end. Also spore size is very close (protologue of *O. orientalis*: 3–4 × 1.5–2 µm, our specimen: 4.0–4.2 × 1.6–1.9 µm). The apothecial margin is only minutely crenulate and hardly bears any glassy processes, while in the *O. orientalis* type, the glassy processes are long and agglutinated, forming distinct teeth at the margin. In the phylogenetic analysis, the teleomorph of *D. yunnanensis* clusters quite far from *D. brochopaga*, although the bootstrap values are low. The ITS similarity between *D. yunnanensis* and *D. brochopaga* (ATCC 96710) is only 87.35, and that between each of other three *D. brochopaga* strains (Genbank accession numbers: AY773456, U51950, U72609) is 86.15, 86.56, and 85.11 respectively, suggesting that *D. yunnanensis* is a species different from *D. brochopaga*. Since we could not identify the teleomorph as a new species based on the limited morphological characters available, we assigned it to *O.* cf. *orientalis*.

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Literature cited

Drechsler C. 1937. Some hyphomycetes that prey on free-living terricolous nematodes. Mycologia 29: 447–552.

Drechsler C. 1954. Some hyphomycetes that capture eelworms in southern states. Mycologia 46: 762–782.

Hagedorn G, Scholler M. 1999. Revaluation of predatory orbiliaceous fungi. I. Phylogenetic analysis using rDNA sequence data. Sydowia 51: 27–48.

Kindermann J, EL-Ayouti Y, Samuels GJ, Kubicek CP. 1998. Phylogeny of the genus *Trichoderma* based on sequence analysis of the internal transcribed spacer region 1 of the rDNA clade. Fungal Genetic Biology 24: 298–309.

Liu XZ, Zhang KQ. 1994. Nematode-trapping species of *Monacrosporium* with special reference to two new species. Mycologia Research 98(8): 862–868.

Pfister DH. 1997. Castor, Pollux and life histories of fungi. Mycologia 89: 1–23.

Scholler M, Hagedorn G, Rubner A. 1999. A reevaluation of predatory orbiliaceous fungi. II. A new generic concept. Sydowia 51: 89–113.

Subramanian CV. 1964 ("1963"). *Dactylella*, *Monacrosporium* and *Dactylina*. J. Indian Bot. Soc. 42: 291–300.

Swofford DL. 2002. PAUP*. Phylogenetic analysis using parsimony, Version 4.0. Sinauer Associates, Sunderland, Massachusetts, USA.

Turner D, Kovacs W, Kuhls K, Lieckfeldt E, Peter B, Arisan-Atac I, Strauss J, Samuels GJ, Börner T, Kubicek CP. 1997. Biogeography and phenotypic variation in *Trichoderma* sect. *Longibrachiatum* and associated *Hypocrea* species. Mycological Research 101: 449–459

White TJ, Bruns T, Lee S, Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: PCR Protocols: a guide to methods and applications. Academic Press, San Diego, CA. pp. 315–322.

Yu ZF, Zhang Y, Qiao M, Baral HO, Weber E, Zhang KQ. 2006. *Drechslerella brochopaga*, the anamorph of *Orbilia* (*Hyalinia*) *orientalis*. Mycotaxon 96: 163–168.

Yu ZF, Zhang Y, Qiao M, Zhang KQ. 2007. *Orbilia dorsalia* sp. nov., the teleomorph of *Dactylella dorsalia* sp. nov. Cryptogamie, Mycologie 28: 289–294.